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- 1) Kirkpatrick, Cell. Mol. Life Sci., 55(3):437-449 (March 1999).
- 2) Kolodner and Marsischky, Curr Opin. Genet. Dev., 9(1):89-96 (Feb. 1999).
- 3) Chamber et al., Mol. Cell. Biol., 16(11):6110-6120 (Nov. 1996).
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- 5) Richardson et al., Biol. Reprod., 62(3):789-796 (March 2000).
- 6) Baker et al., Nat. Genet., 13(3):336-342 (July 1996).
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- 13) Prelle et al., Cells Tissues Organs, 165(3-4):220-236 (1999).

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The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid

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The mismatch repair system is the major barrier to genetic recombination during interspecific sexual conjugation in prokaryotes. The existence of this anti-recombination activity has implications for theories of evolution and the isolation of species. To determine if this phenomenon occurs in eukaryotes, the effect of a deficiency of mismatch repair on the meiotic sterility of an interspecific hybrid of *Saccharomyces cerevisiae* and the closely related species *Saccharomyces paradoxus* was examined. The results demonstrate that the rare viable spores from these hybrids have high frequencies of aneuploidy and low frequencies of genetic exchange. Hybrids lacking the mismatch repair genes *PMS1* or *MSH2* display increased meiotic recombination, decreased chromosome non-disjunction and improved spore viability. These observations are consistent with the proposal that the mismatch repair system is an element of the genetic barrier between eukaryotic species. We suggest that an anti-recombination activity during meiosis contributes towards the establishment of post-zygotic species barriers.

Keywords: meiosis/mismatch repair/non-disjunction/recombination/speciation

Introduction

Genetic recombination is dependent on the formation of a near-perfectly paired heteroduplex joint molecule containing complementary strands from two homologous DNA duplexes. Reduced homology between substrate molecules decreases the efficiency of recombination. A striking example of this can be seen during interspecific crosses between *Escherichia coli* and *Salmonella typhimurium* (Rayssiguier *et al.*, 1989). The genomes of these two bacterial species are diverged by ~16%. At this level of heterology, the frequency of recombination during conjugational crosses is reduced by up to five orders of magnitude, relative to intraspecific crosses. The barrier to recombination is largely dependent on the activity of the mismatch repair system (Rayssiguier *et al.*, 1989; Matic *et al.*, 1995). The 'disrupted species barrier' and 'chromosomal instability' (Petit *et al.*, 1991) phenotypes seen in bacterial mismatch repair mutants are thought to result from a failure to prevent interactions between homeologous (closely related, but non-identical) DNA sequences. This process has been termed anti-recombination, although

its molecular basis remains unclear. The existence of this activity has led to the proposal that the mismatch repair system is involved in controlling the fidelity of genetic exchanges. By permitting only crossovers between truly homologous sequences, such a process would suppress ectopic interactions between dispersed homeologous sequences and thereby avoid potentially lethal chromosome rearrangements. Hence, the recognition of mismatches in duplex DNA may play a role in maintaining the structural integrity of chromosomes.

Many of the elements of the long-patch mismatch repair system that are believed to be involved in anti-recombination have been conserved throughout evolution. Multiple homologues of the bacterial genes *MutS* and *MutL* have been identified in organisms ranging from yeast to man. To date, six *MutS* and three *MutL* homologues have been identified in *Saccharomyces cerevisiae* (reviewed by Kolodner, 1995; Modrich and Lahue, 1996). *PMS1* (Kramer *et al.*, 1989) and *MSH2* (Reenan and Kolodner, 1992a,b) are yeast homologues of the bacterial mismatch repair genes *MutL* and *MutS*, respectively. A dramatic increase in the frequency of the post-meiotic segregation of genetic markers is observed in *pms1* and *msh2* mutants (Williamson *et al.*, 1985; Reenan and Kolodner, 1992b). This is indicative of unrepaired heteroduplex, suggesting a role for these genes in the process of gene conversion. *pms1* and *msh2* mutants also have a mutator phenotype similar to that of bacterial mismatch repair mutants. This reflects a deficiency in repair of DNA synthesis errors and spontaneous DNA lesions (Williamson *et al.*, 1985; Reenan and Kolodner, 1992b). Both gene products have also been shown to form part of a ternary complex that assembles *in vitro* at mismatched base pairs in duplex DNA (Prolla *et al.*, 1994).

Anti-recombination has profound implications for the process of meiosis. During meiotic prophase, the formation of a physical connection between homologues, in the form of a crossover, allows correct orientation on the meiosis I spindle. This ensures the faithful disjunction of chromosomes to produce viable, haploid gametes (reviewed by Hawley, 1988). Mutations that reduce or abolish meiotic crossing-over cause low spore viability, presumably due to extensive chromosomal non-disjunction (reviewed by Roeder, 1990). In yeast, as in bacteria, reduced chromosomal identity (~10–30% DNA sequence divergence) acts as a barrier to recombination and dramatically reduces exchange between homologues during meiosis (Nilsson-Tillgren *et al.*, 1981, 1986; Hawthorne and Philippsen, 1994; S.R. Chambers, N. Hunter and R.H. Borts, in preparation). Radman and co-workers have proposed that the repression of recombination between homeologous chromosomes during meiosis may lead to the reproductive isolation of populations in the form of sterility (Radman and Wagner, 1993; Matic *et al.*, 1995).

To test this proposal, a hybrid of the bakers yeast *S.cerevisiae* and its sibling species *Saccharomyces paradoxus* has been utilized. *Saccharomyces paradoxus* (also described as *Saccharomyces douglasii*: Herbert *et al.*, 1988; Naumov and Naumova, 1990; Adjiri *et al.*, 1994; Hawthorne and Philippsen, 1994) is the closest relative of *S.cerevisiae* isolated to date. Electrophoretic karyotyping and hybridization analysis reveal that the genomes of the two species are very similar in terms of chromosome number, size and the location of genes (Naumov *et al.*, 1992). The weak hybridization of many cloned *S.cerevisiae* genes with *S.paradoxus* chromosomes demonstrates that DNA divergence exists between the two species. From the limited DNA sequence data available, divergence has been estimated to be ~11% and ~20% in coding and non-coding regions, respectively (Herbert *et al.*, 1988; Adjiri *et al.*, 1994). A hybrid of *S.cerevisiae* and *S.paradoxus* therefore comprises genome-wide homeology, but appears to lack major structural differences in karyotype. The current work examines meiotic recombination and chromosome disjunction in this hybrid and the effects of mutations in the mismatch repair genes *PMS1* and *MSH2* on these processes.

Results

Experimental rationale

The model depicted in Figure 1 predicts that meiosis in nuclei containing divergent parental genomes will be associated with both low frequencies of reciprocal exchange and high frequencies of chromosome non-disjunction. This will lead to low viability of the meiotic products (reduced fertility). In the absence of mismatch recognition, crossovers will be permitted between homeologous chromosomes, disjunction will be improved and a greater number of viable, euploid gametes will be produced.

The *S.cerevisiae*/*S.paradoxus* hybrid

A wild-type, homothallic isolate of *S.paradoxus*, N17 (Naumov, 1987), has been engineered into a genetically tractable organism (see Materials and methods). Hybrids of N17 and the *S.cerevisiae* strain Y55 (McCusker and Haber, 1988) produce only 1% viable spores (Tables I and II, strain NHD47). Many of these have abnormal cell and colony morphologies and are slow-growing, often producing only a micro-colony. This sterility has been noted in similar hybrids (Hawthorne and Philippsen, 1994) and forms the basis of the biological species definition of yeast taxonomy (Naumov, 1987).

The low spore viability of the hybrid is expected to be associated with high rates of chromosome non-disjunction. To test this prediction, meiotic chromosome non-disjunction was monitored by physical analysis of the karyotypes of random spores. Separation of the yeast chromosomes by Clamped Homogeneous Electric Field (CHEF) gel electrophoresis allows the assignment of disomy for the 10 smallest *Saccharomyces* chromosomes (Figure 2). Chromosome V is always assumed to be monosomic because spores are selected on medium containing canavanine, which demands the expression of the recessive drug resistant allele, *can1*. In the hybrid NHD47, the frequency of disomy is high for all nine of the chromosomes analysed

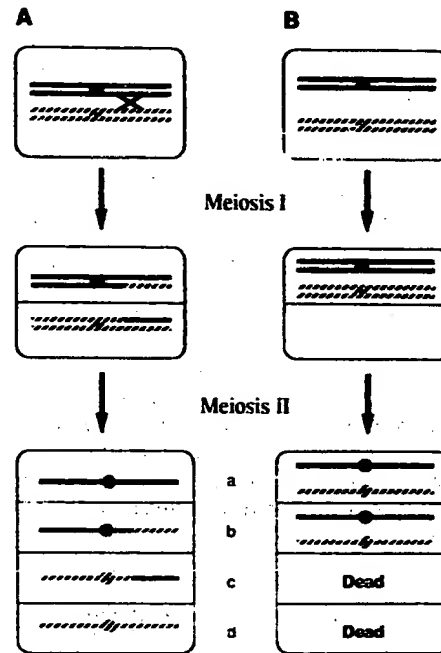


Fig. 1. A model of the biological consequences of anti-recombination during meiosis. (A) Homologous chromosomes recombine and undergo crossing-over. The homologues become physically connected by a chiasma and consequently orientate correctly on the meiosis I spindle. Correct disjunction in the first division is followed by an equational division to produce four euploid spores. Spores b and c contain recombinant chromosomes. (B) The mismatch repair proteins will prevent crossing-over between homeologous chromosomes. Apposition of the centromeres is not ensured and the resultant univalents segregate randomly with respect to each other at meiosis I. If both univalents attach to the same spindle, non-disjunction will result. After meiosis II, two disomic and two nullisomic spores will be produced. None of the chromosomes will be recombinant. The nullisomic cells lack essential genetic information and are dead. The disomic cells contain unbalanced genomes and may have reduced fitness.

(Table III) with the exception of chromosome VI, which is always monosomic (see Discussion). Non-disjunction rates are up to 500-fold higher than that of a *S.cerevisiae* intraspecific diploid. The meiosis I non-disjunction rates of chromosomes IV and XI have been measured by a genetic analysis of random spores in a *S.cerevisiae* Y55 strain at 1.4×10^{-4} and 5.0×10^{-4} per meiosis, respectively (F.E. Pryde and E.J. Louis, unpublished data). Chromosome II exhibited the highest rate of non-disjunction in the wild-type hybrid at 2.7×10^{-1} per meiosis. The distribution of disomes, shown in Figure 3, closely fits that expected from an average non-disjunction rate of 12.2% for the nine chromosomes examined (see Materials and methods). If this calculation is extended to all 16 *Saccharomyces* chromosomes, we expect 12.5% of spores to have no disomes, 27.7% to have one, 22.9% two and 36.9% to have three or more disomes.

The primary cause of chromosome non-disjunction in the interspecific yeast hybrid is proposed to be low frequencies of genetic exchange. To ascertain recombination frequencies, random spores were monitored for recombination in four genetic intervals: *HIS4-LEU2* and *LEU2-MAT* on chromosome III, *TRP1-ADE8* on IV and *CYH2-MET13* on VII (Table IV). The frequencies of

Table I. Strains used in this study

Strain	Genotype	Reference
Y55	<i>S.c. HO</i> wild-type	McCusker (1988)
N17	<i>S.p. HO</i> wild-type	Naumov (1990)
Y55-518	<i>S.c. leu2-1 MATα hoΔPst ura3-<i>nco</i> arg4-1 HIS6 Δpms1</i>	this study
Y55-512	<i>S.c. leu2-1 MATα hoΔPst ura3-1 arg4-1 his6-1 Δpms1</i> <i>S.c. leu2-1 MATα ho-ochre ura3-<i>nco</i> arg4-1 HIS6 msh2::LEU2</i>	this study
NHPD1	<i>S.p. MATα hoΔPst CAN1 ura3-1 LYS5 cyh2-1 pms1Δ::URA3</i>	this study
NHPD2	<i>S.p. MATα hoΔPst can1-1 ura3-1 lys5-1 CYH2 pms1Δ::URA3</i> <i>S.p. MATα hoΔPst CAN1 ura3-1 LYS5 cyh2-1 msh2::URA3</i>	this study
NHD50	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i></i> <i>S.c. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-<i>nco</i></i>	this study
NHD53	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i> pms1Δ::URA3</i> <i>S.c. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-<i>nco</i> pms1Δ::URA3</i>	this study
NHD95	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i> msh2::URA3</i> <i>S.c. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-<i>nco</i> msh2::URA3</i>	this study
NHD47	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i></i> <i>S.p. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-1</i>	this study
NHD45	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i> pms1Δ::URA3</i> <i>S.p. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-1 pms1Δ::URA3</i>	this study
NHD94	<i>S.c. his4-RI leu2Δ MATα hoΔPst trp1-hsu36 ade8-1 met13-4 CYH2 can1 ura3-<i>nco</i> msh2::URA3</i> <i>S.p. HIS4 LEU2 MATα hoΔPst TRP1 ADE8 MET13 cyh2-1 CAN1 ura3-1 msh2::URA3</i>	this study

Abbreviations: *S.c.*, *Saccharomyces cerevisiae*; *S.p.*, *Saccharomyces paradoxus*. All *S.c.* and *S.p.* strains are isogenic to the wild-type isolates, Y55 and N17, respectively. Strains were constructed as described in Methods and materials.

recombination are reduced 10.8- to 73.3-fold relative to the intraspecific control *S.cerevisiae* diploid (NHD50). These frequencies should be considered minimal estimates because segregants that are recombinant and disomic for the chromosome assayed, will always be scored as being non-recombinant. However, it is expected that chromosomes that have undergone reciprocal exchange will disjoin correctly. The *TRP1* to *ADE8* interval demonstrates a profound reduction in map distance. This large genetic interval is 270 cM in *S.cerevisiae* (Mortimer *et al.*, 1992). In the hybrid the markers are tightly linked, with a map distance of ~2 cM.

Mismatch repair-deficient hybrids

To examine the effect of the mismatch repair system on meiosis in *S.cerevisiae*/*S.paradoxus* hybrids, we disrupted the *PMS1* and *MSH2* genes in haploids of both species to produce the hybrid diploids NHD45 and NHD94.

Spore viability is significantly improved in the *pms1* and *msh2* hybrids, by 6.1- and 8.7-fold respectively (Table II). Moreover, the accumulation of haplo-lethal mutations due to the mutator phenotypes of *pms1* or *msh2* produce 21% and 15% spore death in intraspecific diploids (Table II, strains Y55-518, NHPD1, Y55-512 and NHPD2). Therefore, correcting for the death induced by mutation, the viability of the hybrids can be estimated to be 7.4- and 10.0-fold greater than that of the wild-type hybrid. The difference between the viability of the *pms1* and *msh2* hybrids is also significant. Additionally, it was noted that viable spores from *msh2* hybrids are less abnormal in colony morphology and are faster-growing, forming fewer micro-colonies. This may be a direct phenotypic manifestation of lower levels of aneuploidy.

Reduced aneuploidy in mismatch repair mutants

The improvement in spore viability in the mismatch repair-deficient hybrids is concomitant with significant reduction:

Table II. Spore viabilities of intraspecific and hybrid yeast diploids

Strain	Genotype	Percentage spore viability
Y55	<i>S.c. wt</i>	97.8 (841/860)
Y55-518	<i>S.c. pms1</i>	80.5 (679/844)
Y55-512	<i>S.c. msh2</i>	84.0 (776/924)
N17	<i>S.p. wt</i>	97.0 (194/200)
NHPD1	<i>S.p. pms1</i>	71.3 (117/164)
NHPD2	<i>S.p. msh2</i>	80.4 (193/240)
NHD47	hybrid wt	1.2 (10/852)
NHD45	hybrid <i>pms1</i>	7.2 (63/880)
NHD94	hybrid <i>msh2</i>	10.2 (147/1440)

Diploids were sporulated and tetrad ascospores dissected. To reduce the spore death caused by the mutator phenotypes of *pms1* and *msh2*, vegetative growth as a diploid was minimized. Haploid strains were mated for only 6 h at 30°C, and the diploids were not selected prior to sporulation. All strains were treated in this way. The spore viability of all three hybrids is significantly different from all intraspecific diploids as determined by a standard normal test ($P < 0.001$). The *pms1* and *msh2* hybrid viabilities are different from the wild-type hybrid ($P < 0.01$ and $P < 0.001$, respectively), and the *msh2* hybrid is different from the *pms1* hybrid ($P < 0.01$).

in disomy. In both mutant hybrids, there is an improvement in the disjunction of all the chromosomes analysed (Table III). In the *pms1* hybrid, the total frequency of disomes is reduced 1.8-fold over the wild-type hybrid. The improvement in disjunction is even greater in the *msh2* hybrid, with a further 1.8-fold reduction in total disomes. This indicates a significant disparity between the *pms1* and *msh2* mutants with respect to chromosome disjunction. In addition, the distribution of disomes among the three hybrid diploids is significantly different (Figure 3). In the wild-type hybrid, only 32% of spores are not disomic for any of the nine chromosomes analysed and nearly 12% contain three or more disomes. By comparison, 70% of the spores from the *msh2* hybrid have zero detectable disomes and no spores contain more than two disomes.

Recombination is increased in *pms1* and *msh2* hybrids

Genetic analysis of random spores from the *pms1* mutant hybrid reveals a 2.3- to 10.0-fold increase in recombinants for the four intervals monitored. As might be expected from the disjunction data, the effect of the *msh2* disruption is greater, producing a 6.0- to 16.5-fold increase in recombinant frequency. Again, these results reflect a significant difference between the two mutant hybrids. No change in recombinant frequency is observed in the *pms1*

and *msh2*, intraspecific *S.cerevisiae* diploids (NHD53 and NHD95), demonstrating that the observed effects are specific to the hybrids. The improved spore viability of the *msh2* hybrid permitted limited 'tetrad analysis' to be performed. Out of 53 tetrads with one or more viable spores, 3.9% had a recombination event in the *HIS4-LEU2* interval; 19.6% between *LEU2* and *MAT*, 41.2% in the *TRP1-ADE8* interval and 3.8% between *CYH2* and *MET13*. These frequencies are not statistically different from those obtained in the analysis of random spores. In addition, bona fide reciprocal events in three of the four intervals analysed (*LEU2-MAT*, *TRP1-ADE8* and *CYH2-MET13*) were represented in the tetrads with two or more viable spores.

Discussion

The meiotic behaviour of an interspecific yeast hybrid satisfies the predictions of the anti-recombination model

In the hybrid between *S.cerevisiae* and *S.paradoxus* the frequencies of meiotic recombinants are reduced to between 1.3 and 8.7% of intraspecific frequencies over four genetic intervals that vary from 11 to 270 cM. The reduction is greatest over the largest region, *TRP1* to *ADE8*. The map distance is reduced ~135-fold, predicting that a crossover in this interval will occur in <1% of meioses. The reduction in recombinants is small when compared with bacterial interspecific crosses. Recombination during conjugational crosses between *E.coli* and *S.typhimurim* is reduced by up to five orders of magnitude compared with the equivalent intraspecific crosses (Rayssiguier *et al.*, 1989). However, Hfr recombination involves the replacement of a continuous block of the recipient genome, a process requiring two distant crossovers. Therefore, the frequency of recovery of conjugational recombinants will be the probability of completing two independent crossovers. Thus, a 300-fold reduction per crossover is equivalent to five orders of magnitude reduction in recombination. Our results are consistent with those of Shen and Huang (1986) who observe a 25- to 300-fold decrease in recombination with substrate homologies of 90-65%. The phage-plasmid recombination assay utilized in this study requires only one reciprocal exchange for the recovery of recombinants. This is more analogous to meiotic crossing-over than a system based on conjugational recombination.

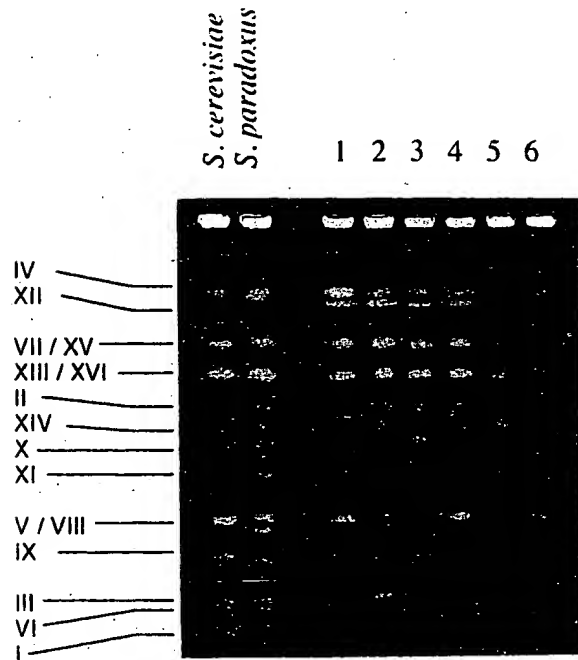


Fig. 2. Chromosome disomy in hybrid segregants. CHEF gel electrophoresis separates the 16 *Saccharomyces* chromosomes into 13-14 bands. The electrophoretic karyotypes of parental haploids Y55 and N17 and six random segregants of the wild-type hybrid, NHD47, are shown. There are three obvious size polymorphisms between the two yeast species: at chromosomes I, VIII and II. Disomy for the 10 smallest chromosomes is assigned by the presence of a doubly intense band or two bands for the polymorphic chromosomes. Disomic chromosomes are as follows: lane 1, chromosome I disome; lane 2, chromosome III disome; lane 3, chromosome IX and X disomes; lane 4, chromosome VIII disome; lane 5, chromosome XIV and II disomes; lane 6, chromosome II disome.

Table III. Frequency of disomes in hybrid segregants

Strain	Percentage of spores with disome								Total
	I	VI	III	IX	VIII	XI	X	XIV	
NHD47 (wt)	18.4 (19/103)	0.0	9.7 (10/103)	12.6 (13/103)	21.4 (22/103)	5.8 (6/103)	13.6 (14/103)	1.0 (1/103)	12.2 (12/103)
NHD45 (<i>pms1</i>)	13.3 (14/105)	0.0	7.6 (8/105)	5.7 (6/105)	7.6 (8/105)	1.9 (2/105)	8.6 (9/105)	0.0	6.6 (7/105)
NHD94 (<i>msh2</i>)	4.0 (4/100)	0.0	6.0 (6/100)	4.0 (4/100)	3.0 (3/100)	5.0 (5/100)	7.0 (7/100)	0.0	3.7 (4/100)

Random segregants were karyotyped by CHEF gel electrophoresis. The total numbers of disomes are significantly different between all three data sets as defined by a standard normal test ($P < 0.01$ to $P < 0.001$) and the individual data sets are different by a non-parametric sign test ($P < 0.05$ to $P < 0.01$). The data sets for chromosomes VIII and II are different between NHD47 and NHD45 ($P < 0.01$ and $P < 0.05$, respectively). Chromosomes I, IX, VII, X and II data sets are different between NHD47 and NHD94 ($P < 0.05$ to $P < 0.001$). The frequency of disomes for chromosomes I and II are significantly different between NHD45 and NHD94 ($P < 0.01$).

The low recombination rates in the hybrid confer a several hundred-fold increase in the frequency of non-disjunction. This should be considered to be a minimum estimate because viable random spores may be under-represented for aneuploidy. Particular combinations of disomes are either lethal or produce slow-growing colonies (Parry and Cox, 1970). In this study chromosome VI was never found to be disomic in over 300 CHEF karyotypes. This could be because VI disomy is lethal. While this is not the case for intraspecific *S.cerevisiae* diploids, in

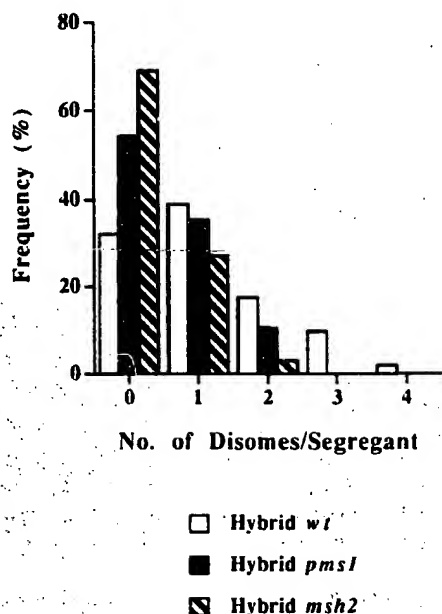


Fig. 3. Distribution of disomes in hybrid segregants. These data represent the same sample of random spores analysed in Table III. No more than four of the nine chromosomes analysed were disomic in any one cell. The observed distributions closely fit that expected from the average rates of disomy per chromosome, calculated from Table III (see Data analysis). The distributions for each strain are different, as determined by a G-test ($P < 0.005$).

which VI disomy is tolerated, it is possible the *S.cerevisiae* and *S.paradoxus* chromosomes are incompatible. Alternatively, it is possible that chromosome VI always disjoints correctly.

The mismatch repair proteins *Pms1* and *Msh2* reduce meiotic homeologous recombination

The *pms1* mutation restores meiotic recombination in the hybrid to between 9 and 27% of intraspecific frequencies. In *msh2* hybrids, the frequency of recombinants is 20–69% of the homologous controls. The increase in *TRP1-ADE8* map distance in the *msh2* hybrid predicts that a crossover will occur in nearly 50% of meioses. However, this is still a low frequency when compared with the intraspecific *S.cerevisiae* interval, which has about five crossovers per meiosis. Recombinants are observed in spores from tetrad dissection at frequencies equivalent to those from random spore analysis. It is important to note that both products of reciprocal exchange events are recovered in the tetrads with two or more viable spores from this analysis. This indicates that most random spore recombinants represent true crossover products. The *pms1* and *msh2* Y55 intraspecific control diploids have no increase in the frequency of meiotic recombination. These controls rule out the possibility that the increase in recombinants in the mutant hybrids is due to a general hyper-recombination phenotype or to marker reversion. We conclude that the mismatch repair system actively inhibits meiotic exchange between highly divergent chromosomes.

The observation that recombination is never fully restored in mismatch repair-deficient hybrids could be due to several factors. Other mismatch repair proteins that inhibit homeologous recombination may still be active in these mutants. Also, the degree of initiation of meiotic recombination may be reduced between homeologous chromosomes. This 'trans effect' of heterozygosity has been observed at two loci in *S.cerevisiae* (Xu and Kleckner, 1995; V.Rocco and A.Nicholas, personal communication). Also, some regions of the chromosomes may be so

Table IV. Meiotic recombination

Strain	Percentage recombinants			
	<i>HIS4-LEU2</i>	<i>LEU2-MAT</i>	<i>TRP1-ADE8</i>	<i>CYH2-MET13</i>
NHD50	18.33	21.67	46.39	11.94
(<i>S.c.</i> wt)	(66/360)	(78/360)	(167/400)	(43/360)
NHD53	17.22	24.72	49.44	11.94
(<i>S.c. pms1</i>)	(62/360)	(89/360)	(178/360)	(43/360)
NHD95	23.8	22.78	47.5	9.17
(<i>S.c. msh2</i>)	(86/360)	(82/360)	(171/360)	(33/360)
NHD47	0.25	2	2	0.25
(hybrid wt)	(1/400)	(8/400)	(8/400)	(1/400)
NHD45	1.75	4.5	13	2.5
(hybrid <i>pms1</i>)	(7/400)	(18/400)	(52/400)	(10/400)
NHD94	4	12	33	3.5
(hybrid <i>msh2</i>)	(16/400)	(48/400)	(132/400)	(14/400)

Random spores were analysed for recombination in the four intervals shown. Map distance in cM is equivalent to the frequency of recombinants. None of the intervals in the control diploids, NHD50, 53 and 95 are statistically different by standard normal tests. Therefore, a pool of these data sets was used for comparison to data from the hybrid diploids. All intervals in the three hybrid diploids are significantly different from the intraspecific controls ($P < 0.001$). The recombinant frequencies in all four intervals in the *pms1* hybrid are statistically different from those in the wild-type hybrid ($P < 0.05$ to $P < 0.001$). Likewise all intervals are different between *msh2* and wild-type hybrids ($P < 0.001$ to $P < 0.0001$). Additionally, the *LEU2-MAT* and *TRP1-ADE8* data sets are different between *pms1* and *msh2* hybrids ($P < 0.001$) and the *HIS4-LEU2* data is suggestive of a difference ($P = 0.056$). The total number of recombinants is also significantly different between the three hybrids ($P < 0.001$).

diverged that homology is no longer recognized at the strand exchange stage of recombination (see also below).

Crossovers ensure disjunction

The correlation between greater crossing-over and decreased aneuploidy indicates that many of the crossovers restored in the mutant hybrids can form functional chiasmata (a cytological manifestation of crossing-over) and thereby ensure disjunction. From a comparison with *S. cerevisiae* recombination-deficient mutants, we propose that the deficiency of recombination is the major reason for non-disjunction in the hybrid. For example, the *med1* mutation (Rockmill and Roeder, 1994), an allele of the *DMC1* gene (Bishop *et al.*, 1992; Rockmill *et al.*, 1995), has a 2-fold decrease in meiotic crossovers, 4.3% chromosome III and 6.6% chromosome VIII disomes and 20% spore viability. This is similar to the *msh2* hybrid, which displays a ~3-fold decrease in exchange, 6% chromosome III and 3% chromosome VIII disomes and 10% spore viability.

It must also be considered that the reduced fidelity of recombination in mismatch repair-deficient hybrids may increase the frequency of crossovers between related, ectopic loci. Ectopic crossovers are known to interfere with homologue disjunction (Lichten *et al.*, 1987) and are likely to produce lethal, unbalanced translocations. One such translocation, giving rise to a unique-sized chromosomal species has been observed in a segregant from the *msh2* hybrid (not shown).

Spore viability is improved in *pms1* and *msh2* hybrids

The increased spore viability in *pms1* and *msh2* hybrid diploids appears to be a direct consequence of improved chromosome disjunction, which in turn is the result of increased frequencies of meiotic recombination. The spore viability of the hybrids is lower than expected from the patterns of disomy observed. The average frequency of disomes in random spores from the *msh2* hybrid is 3.7% per chromosome. The observed frequency of 70% spores with no disomes, for the nine chromosomes examined, closely fits the expected frequency. If this rate of disomy is assumed for all 16 *Saccharomyces* chromosomes, the expected number of spores with no disomes is 55%. Therefore, the minimum expected spore viability for the *msh2* hybrid is 55%. The fact that spore viability is not restored to this level indicates that other factors probably contribute to the meiotic sterility of the *S. cerevisiae*/*S. paradoxus* hybrid. The observation that some *S. paradoxus* chromosomes are haplo-insufficient in an otherwise *S. cerevisiae* genetic background (S.R. Chambers, N. Hunter and R.H. Borts, unpublished observations) suggests that chromosomal rearrangements or incompatibilities may be present, and could contribute significantly to spore inviability. In summary, an active mismatch repair system reduces meiotic exchange between divergent chromosomes, increases their rate of non-disjunction and reduces spore viability.

Processing of mismatched recombination intermediates

How the mismatch repair system processes mismatched recombination intermediates at the molecular level is not

clear. Several models have been proposed. The 'killer mechanism' causes the destruction of intermediates (Doutriaux *et al.*, 1986) and could potentially lead to chromosomal loss. Mismatch-repair-induced recombination may lead to chromosomal rearrangement or loss (Borts and Haber, 1987; Borts *et al.*, 1990). Finally, the anti-recombination model (Radman, 1989) and the similar 'heteroduplex rejection' model (Alani *et al.*, 1994) propose that intermediates are aborted via disassembly, or resolution without exchange. From the data presented in this study, none of these possibilities can be excluded. However, increases in non-disjunction are not necessarily predicted by the first two models. Therefore, the data presented here are most consistent with an anti-recombination mechanism.

Two observations suggest that recombination intermediates between homeologous sequences are disrupted at an early stage, prior to the formation of stable heteroduplex junctions. First, individual components of the bacterial mismatch repair system can block *in vitro* homeologous strand exchange catalysed by the *E. coli* RecA protein (Worth *et al.*, 1994). Second, double Holliday junction recombination intermediates that have been detected during meiotic prophase I, in *S. cerevisiae* (Collins and Newlon, 1994; Schwacha and Kleckner, 1994, 1995) were not observed to form between homeologous chromosomes (Collins and Newlon, 1994).

Mutations in the mismatch repair genes *MSH2* and *MSH3* (New *et al.*, 1993) have been shown to increase the frequency of mitotic homeologous recombination between substrates with 73% identity in *S. cerevisiae* (Selva *et al.*, 1995). Mutation of *PMS1* had no significant effect on recombination, as observed by other workers utilizing mitotic recombination assays with similarly diverged substrate DNA (~80% identity) (Bailis and Rothstein, 1990; Resnick *et al.*, 1992). In another mitotic study, a 10-fold effect of *pms1* was observed with 92% identical substrates (Datta *et al.*, 1996). In the data presented here, *pms1* produces up to a 10-fold enrichment in meiotic recombinants. The effect of *msh2* is significantly greater, not only in terms of recombination, but also for disomy and spore viability. This observation and data to be presented elsewhere (N. Hunter and R.H. Borts; S.R. Chambers, N. Hunter and R.H. Borts, in preparation) lead us to suggest that the method of processing recombination intermediates depends upon the degree of divergence between the participating molecules. At relatively high levels of divergence (10–30%) *MSH2* appears to have a greater role than *PMS1* in preventing homeologous recombination.

From the known biochemical properties of the *E. coli* MutS and *S. cerevisiae* Msh2 proteins (reviewed in Modrich and Lahue, 1996), it is assumed that DNA divergence will be recognized when mismatches form in heteroduplex DNA. A number of features of meiotic homeologous recombination follow from this assumption. The fact that moderate frequencies of recombination are observed in *pms1* and *msh2* hybrids suggests that the induction of meiotic recombination is still high. Also, consistent with the *in vitro* properties of *E. coli* RecA protein (DasGupta and Radding, 1982), high densities of mismatches are not normally inhibitory to strand exchange *per se* in yeast. However, very high divergence (>30%

mismatches) may act as a structural barrier to strand exchange.

A role for the mismatch repair system in speciation

We suggest that the mismatch repair system plays a role in the evolution and speciation of eukaryotic organisms. The extent to which mismatch repair contributes to the process of speciation can only be speculated upon. The effects reported in this study are likely to occur when two previously isolated populations meet in a hybrid zone. The mismatch repair system can therefore be thought to reinforce the isolation of the two populations. The reduced recombination, and hence gene flow, between diverging chromosomes will cause them to become increasingly isolated genetic units. This hypothesis is supported by observations made with trisomics and hexaploid nuclei containing both homologous and homeologous chromosome pairs, in which homologues preferentially recombine and segregate from each other (Nilsson-Tillgren *et al.*, 1981, 1986; Williams *et al.*, 1993; S.R.Chambers, N.Hunter and R.H.Borts, unpublished observations). A role for mismatch repair during the incipient stages of speciation can also be envisaged. Low levels of heterology may lead to chromosomal rearrangement between repeated sequences, via a mismatch repair-induced recombination mechanism (Borts and Haber, 1987; Borts *et al.*, 1990). As the divergence between two populations increases, the degree of anti-recombination and chromosome non-disjunction will become ever greater and fertility will be reduced. This process could contribute significantly to the establishment of post-zygotic species barriers.

Materials and methods

Strains

All *S.cerevisiae* and *S.paradoxus* strains used in this study are isogenic derivatives of Y55 and N17 respectively. Genotypes are described in Table I. The *ho-ochre* mutation was isolated by UV-mutagenesis (E.Louis, unpublished data) but was found to have a slightly leaky phenotype. Subsequently, heterothallic strains were obtained by deleting a 100 bp *Pst*I fragment of the coding sequence of the *HO* gene. The *Δpms1* mutation is a 2.6 kb deletion of the *PMS1*-coding sequence (Kramer *et al.*, 1989). Both *hoΔPst* and *Δpms1* were cloned into a *URA3* selectable, integrative vector and introduced via two-step gene replacement. The *pms1Δ::URA3* mutation is a *URA3* replacement of 2.6 kb of the *PMS1* coding sequence (Lichten *et al.*, 1990). *msh2::LEU2* is an insertion of *LEU2* at a *Sna*BI site of the wild-type gene in plasmid pII-2 (Reenan and Kolodner, 1992b). Both were introduced by one-step gene transplacement. *his4-R1*, *trp1-hsu36* and *ura3-nc0* are restriction site fill-in mutations. *lenΔ* is a deletion of most of the *LEU2* coding region. All three mutations were introduced by two-step gene replacement. Other auxotrophic and drug resistance markers were spontaneous or UV-induced (McCusker and Haber, 1988). All transformations were verified by Southern blot analysis (Southern, 1975; Sambrook *et al.*, 1989) using the digoxigenin, non-radioactive system as recommended by the manufacturer (Boehringer Mannheim).

Genetic procedures

Yeast manipulations and media were carried out as described by Rose *et al.* (1990). Strains were grown at 30°C on YPD and synthetic complete media lacking one or more amino acids. Sporulation was performed at room temperature on KAc plates containing 2% potassium acetate, 0.22% yeast extract, 0.05% glucose, 2.5% agar and 0.09% complete amino acid mixture. Dissected tetrads were grown for 3–7 days at 30°C. Germination was scored microscopically after 3 days. Only spores that formed micro-colonies were scored as being viable. Random spores were prepared as described (Lichten *et al.*, 1987) and grown on synthetic complete medium lacking arginine and containing cycloheximide

(10 mg/l) and canavanine (40 mg/l) for 3–6 days at 30°C. One- and two-step gene replacements were performed as described (Rose *et al.*, 1990). Yeast transformation was carried out using a modification of the lithium acetate method (Gietz, 1992).

Karyotyping of segregants

Random spore segregants were karyotyped as described (Naumov *et al.*, 1992). Disomy was assigned via band intensity or the presence of two bands for co-migrating and polymorphic chromosomes, respectively.

Data analysis

Data sets were analysed using the standard normal, non-parametric sign and G-tests as described (Sokal and Rohlf, 1969). The G-test is an equivalent to the χ^2 contingency test. Values of $P < 0.05$ were considered significant. Expected distributions of disomes were calculated using the average disomy frequencies in a binomial expansion involving 9 or 16 chromosomes.

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